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Effect of *cis* and *trans* unsaturated fatty acids on the transport properties of *Salmonella typhimurium*

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Abstract. The transport of α-methyl-D-glucoside and two aminoacids, L-phenylalanine and L-leucine by a temperature sensitive fatty acid requiring mutant of *Salmonella typhimurium* was studied under conditions of supplementation with *cis* or *trans*-unsaturated fatty acids. The results of such experiments definitely establish a relationship between the fatty acids composition of the membrane and the transport property of the cells. Cells grown in the presence of *trans*–unsaturated fatty acids cannot transport so efficiently as compared to the *cis*-unsaturated fatty acid-grown cells except linolelaidic acid, a *trans-trans*-unsaturated fatty acid. Protein: phospholipid ratio of the membrane also varies significantly under such conditions. The affinity of L-phenylalanine transport carrier for the substrate changes remarkably in cells grown in the presence of different *cis* or *trans*-unsaturated fatty acids and indicate the possible role of membrane lipids in membrane assembly as well as regulation of the activity of L-phenylalanine transport system.

Keywords. Transport; fatty acid auxotroph; *cis* and *trans*-unsaturated fatty acid.

Introduction

The role of membrane lipids in various membrane associated biological function has become quite evident from the reports in the literature (Endo *et al.,* 1969; Milner and Kaback, 1970; Higashi and Strominger, 1970; Kundig and Roseman, 1971; Jakovcic *et al.,* 1971; Schneider and Kenedy, 1973; Silbert, 1975; Esfahani *et al.,* 1977; Mandal *et al.,* 1978; Cronan, 1978; Deb *et al.,* 1986). In most of the investigations lipid composition of the membrane has been changed by altering growth temperature and in a fewer cases by supplementing different fatty acids in the media. However, no direct comparison of the effect of *cis* and *trans*-fatty acid supplementation has been carried out to investigate the effect of membrane fluidity on membrane function except the results reported from our laboratory (Deb *et al.,* 1986). It has been reported from this laboratory that many of the membrane functions and especially the transport of exogenous uridine is very much dependant on the fatty acid composition of the membrane and particularly on the ratio of saturated and unsaturated fatty acids. As reported earlier (Deb *et al.,* 1986) the lipid composition of the cell membrane has been altered by supplying different fatty acids to the growth media of a temperature sensitive fatty acid biosynthetic mutant, fabB2 of *Salmonella typhimurium* which cannot synthesise fatty acid at 37°C and thus requires exogenous fatty acids for growth at non-permissive temperature (Hong and Ames, 1971). To study the efect of membrane fluidity on cellular transport processes the cells were supple-

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Abbreviations used: α-MG, α-Methyl-D-glucoside; MM, minimal medium; DPH, l,6-diphenyl-l,3,5, hextriene; ANS, 1,8-anilino naphthalene sulphonic acid.

mented with either *cis* or *trans*-unsatur&ted fatty acid. The fatty acids used in the study were so chosen that they vary in chain length, degree of unsaturation and steric configuration (table 1). The cellular transport process being one of the most well characterised membrane functions, the transport of 3 substances, α-methyl-D-glucoside (α-MG), L-leucine and L-phenylalanine was chosen for study. Transport of the different solutes was studied to explore whether different transport carriers require different limpid environment.

Table 1. Fatty acids used.

Common name	Systematic name	No. of carbon atoms	Formula
Palmitoleic	*cis*-Δ^9-Hexadecenoic	16	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$
Palmitelaidic	*trans*-Δ^9-Hexadecenoic	16	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$
Oleic	*cis*-Δ^9-Octadecenoic	18	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
Elaidic	*trans*-Δ^9-Octadecenoic	18	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
Linoleic	*cis, cis*-Δ^9, Δ^{12}-Octadecadienoic	18	$CH_3(CH_2)_4(CH=CHCH_2)_6COOH$
Linolelaidic	*trans, trans*, Δ^9, Δ^{12}-Octadeca-dienoic	18	$CH_3(CH_2)_4(CH=CHCH_2)_6COOH$

Materials and methods

Chemicals

[^{3}H]-L-Leucine (5200 mC/m mol), [^{14}C]-L-phenylalanine (10mC/mmol), [^{14}C]-α-methyl-D-glucoside (4·5 mC/m mol) were obtained from Bhabha Atomic Research Centre, Bombay. 2,5-Diphenyloxazole (PPO) and 1,4-di (2-5-phenyloxazolyl benzene) (POPOP) were purchased from Amersham/Searle Corporation, USA. The fatty acids used in the study were from Sardary Research Laboratories, London, Ontario, Canada and obtained as generous gifts from Proof. B. D. Sanwal, Department of Biochemistry, University of Western Ontario, Canada. All other chemicals were of analytical grade.

Bacterial strain

S. typhimurium fabB2, a fatty acid biosynthetic mutant, used in this study was a gift from Prof. B. Ames of the Department of Biochemistry, University of California, Berkeley, California, USA. The mutant is deficient in *β*-ketoacyl carrier protein synthetase (Hong and Ames, 1971). The strain LT2 was originally obtained from Prof. M. Levine, Department of Human Genetics, Ann Arbor, Michigan, USA.

Growth media and growth of fabB2

S. typhimurium was normally grown in minimal medium (MM) the composition of which was as described by Chakravorty (1970). The composition of the mineral base

medium was as described by Hoffee *et al.* (1964). Conditions for growth in fatty acid supplemented media was as described earlier (Deb *et al.,* 1986).

Protein estimation

Protein was determined by the method of Lowry *et al.* (1967). Micro-determination of protein was done using Coomassie blue as described by Braford (1976). Bovine serum albumin was used as standard in both cases.

Estimation of phospholipids

Total phospholipids from membrane vesicles were extracted by the method of Bligh and Dyer (1959) and inorganic phosphate was estimated as per the method of Chen *et al.* (1956).

Transport of α-MG, L-leucine and L phenylalanine

Transport of exogenous (α-MG), [^{14}C]-L-leucine and [^{14}C]-L-phenylalanine was measured according to the method of Khandekar *et al.* (1975) with the following modifications. The fabB2 cells were grown at 37°C for 4–5 generations in MM containing 0·2% glycerol, 004% Brij-58 and 0·4mM requisite fatty acid. LT2 cells used as control were also grown under similar condition but without any fatty acid. Subsequently [^{3}H]-leucine (10 nmol, 5·9 × 10^5 counts/min) or [^{14}C]-L-phenyl-alanine (12 nmol, 2·2×10^5 counts/min) or [^{14}C] -α-MG (100 nmol, 1·5 × 10^5 counts/ min) was added per ml of cell suspension for transport studies. Concentration of α-MG, L-phenylalanine and L-leucine used in the incubations were the saturating amounts as determined experimentally under the present conditions. Counting was done in Mark II Liquid Scintillation Counter of Nuclear Chicago and Company or LKB Rack Beta Counter using toluene based scintillation fluid.

Fluorescence polarisation studies

Fluorescence spectra were recorded on Perkin-Elmer LS-5 Spectrofluorometer with 10 nm excitation and emission band pass. Fluorescence studies were carried out with membrane vesicles prepared from cells grown in *cis* or *trans*-unsaturated fatty acid supplemented media. 1,6-Diphenyl-1,3,5 hextriene (DPH) was added from 2 mM solution in tetrahydrofuran to the sample to achieve a final concentration of 4 μm so that tetrahydrofuran was less than 0·001%. For distribution of DPH between the hydrophobic and hydrophilfic regions of the membrane the vesicles were incubated with DPH for 15 min at 37°C. Measurements were recorded using λ (excitation) = 355 nm and λ (emission) = 430 nm.

Results and discussion

Effect of different fatty acid supplementation on the fluidity of membrane

It is well-known that the fluidity of the membrane is determined by the nature of the fatty acyl moiety of the cell membrane. To alter the fluidity of the membrane,

cells were grown in the presence of either *cis* or *trans*-unsaturated fatty acids. Fatty acid analysis of such cells indicated that cells grown in the presence of *trans*-unsaturated. fatty acids possess higher proportion of unsaturated fatty acid (Deb *et al.,* 1986). Fluidity of such membranes was probed with lipid specific probe DPH. When membrane is treated with DPH, it goes to lipid bilayer and the value of fluorescence polarisation of DPH (*'P'*) reflects the total fluidity of the membrane. Results presented in table 2 clearly indicate that in case of each pair of *cis* and *trans*-isomer (except that of linoleic and linolelaidic acid grown cells) the fluidity of *trans*-fatty acid supplemented membrane is less than those of the *cis* unsaturated fatty acid supplemented cells (as evident from higher polarisation value). Membrane vesicles from cells grown in the presence of either linoleic or linolelaidic acid exhibited similar fluorescence polarisation value. This is in conformity with the physical properties of the fatty acids used to supplement the media. Preliminary experiments with 1,8-anilino naphthalene sulphonic acid (ANS) also support the results obtained with DPH (ANS data not presented, will be published in detail later).

Table 2. DPH polarisation values of the membrane vesicles of fabB2 supplemented with different fatty acids.

Fatty acid supplementation	Temperature °C	'p' (polarisation value)
—	30	0·27
Oleic	37	0·23
Elaidic	37	0·32
Palmitoleic	37	0·24
Palmitelaidic	37	0·28
Linoleic	37	0·28
Linolelaidic	37	0·29

Effect of different cis or trans fatty acid supplementation on cellular transport process

The effect of supplementation with *cis* or *trans*-unsaturated fatty acids on the transport of α-MG, L-phenylalanine, L-leucine was studied (results of experiment have been presented in figures 1-3). In these experiments the *trans, trans*-unsaturated fatty acid linolelaidic acid has also been used as it resembles *cis*-unsaturated fatty acid in configuration and physical properties. In all the cases (figures 1-3) the transport as well the steady state level in *trans*-unsaturated fatty acid grown cells are invariably less than those in *cis*-unsaturated fatty acid grown cells although degree of diference varies from one transport system to another. This situation was not expected in case of linoleic-linolelaidic acid pairs (figures 1C, 2C and 3C) as linolelaidic acid resembles *cis*-unsaturated acid in physical properties. Moreover, membrane vesicles prepared from linolelaidic acid supplemented cells are as fluid as the vesicles prepared from other *cis*-unsaturated fatty acid grown cells (table 2). Even then the transport in such cells is comparatively low. Thus for transport processes to function efficiently either proper fluidity of the membrane or correct lipid environment or both are necessary. Cells grown in the presence of elaidic acid are very inefficient in transporting phenylalanine. The intracellular concentration of the exogenous phenylalanine is almost the same as that of the extracellular medium

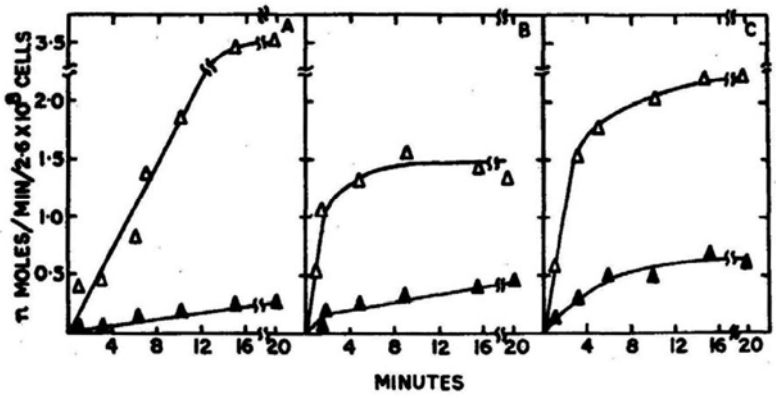

Figure 1. Transport of α-methyl-D-glucoside by fabB2 grown at 37°C in media supplemented with *cis* or *trans*-isomers of 3 different fatty acids. The condition of growth was the same as that described in 'materials and methods'. *Cis* or *trans*-fatty acids used were either palmitoleic or paimitelaidic (A); oleic or elaidic, (B); and linoleum or linolelaidic (C). (Δ), *cis*-unsaturated fatty acid; (▲), *trans*-unsaturated fatty acid.

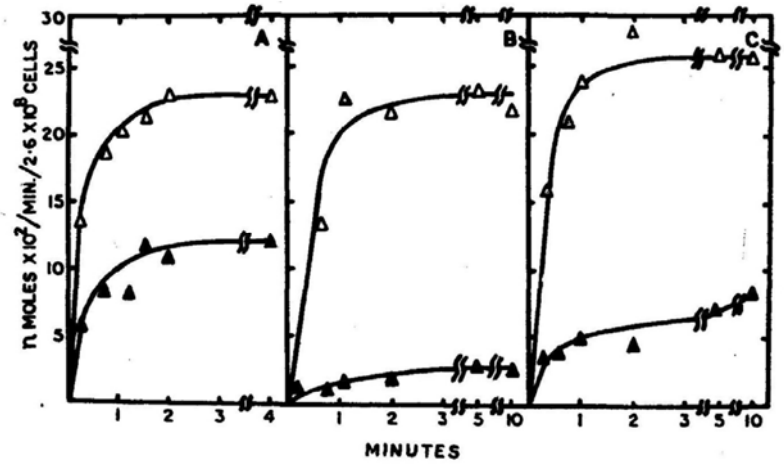

Figure 2. Transport of L-phenylalanine by fabB2 grown at 37 C in media supplemented with *cis* or *trans*-isomer of 3 different fatty acids. Experimental conditions are as described under 'materials and methods'. Symbols are as in figure 1.

(figure 2B). Our results indicate that the fluidity of the membrane is not the sole factor which determines that activity of the amino acid transport carriers.

Effects of fatty acid composition of the cell membrane on the affinity of the L-phenylalanine transport system for phenylalanine

The results presented above clearly indicate that the cells grown in the presence of *trans*-unsaturated fatty acid exhibit very slow rates of transport of -MG, leucine

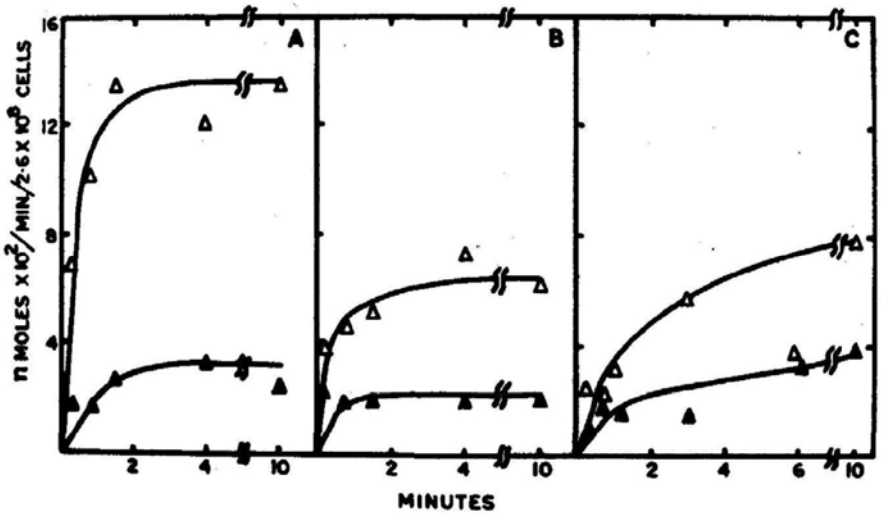

Figure 3 Transport of L-leucine by fabB2 grown at 37°C in media supplemented with *cis* or *trans* isomers of 3 different fatty acids. Experimental conditions are described under 'materials and methods'. Symbols are as in figure 1.

and phenylalanine. This may be due to inefficiency functioning of the transport carriers either due to rigidity of the cell membrane or absence of specific lipids in the near vicinity of the transport carriers in the membrane or lesser number of transport carriers per cell. In order to ascertain the above, the affinity of the L-phenylalanine transport system for its substrate L-phenylalanine has been determined in cells grown in the presence of different fatty acids. The V_{max} and K_m values for L-phenylalanine transport calculated from Lineweaver-Burk plot (figures not presented) are shown in table 3. The values for V_{max} obtained with *cis*-unsaturated fatty acid grown cells are almost half of the values obtained with LT2 (wild) cells. The values of V_{max} with palmitoleic and palmitelaidic *(cis-trans* pairs) acids grown cells are almost the same and half of the value of the control but the K_m varies to a great extent (almost 25 fold). The affinity of the phenylalanine transport carrier for the substrate changes remarkably in cells grown in the presence of palmitelaidic acid. This observation also confirms the idea that not only the fluidity but also the lipid composition (environment) is important in the functioning of at least some of the amino acid transport carriers. Further, the lipid environment may change the affinity of the transport carriers to their respective substrates. On the other hand, the K_m values obtained with oleic and elaidic acid grown cells (other *cis-trans* pair) are not much different while V_{max} differs 30 fold. The elaidic acid grown cells appear to have less transport carriers than all the other types of cells. Elaidic acid grown cells cannot utilise D-lactate as the sole carbon source (unpublished results) and also respire at a reduced rate (Deb *et al.,* 1986). These may be explained by assuming that under condition of elaidic acid supplementation the membrane becomes deficient in proteins. Either less membrane proteins are synthesised or assembled into the membrane. Lipid protein ratio of different membrane vesicles as presented in table 4 provides further support in this line. When the cells are grown in the presence of

Table 3. The V_{max} and K_m values for L-phenylalanine transport system of fabB2 cells grown in the presence of different fatty acids.

Supplementation	V_{max} (n mol/min/2·6 × 10^8 cells)	K_m
Control*	0·75	$1·5 \times 10^{-6}$ M
Palmitoleic	0·43	$8·3 \times 10^{-7}$ M
Palmitelaidic	0·40	$2·0 \times 10^{-5}$ M
Oleic	0·45	$7·7 \times 10^{-7}$ M
Elaidic	0·014	$3·3 \times 10^{-7}$ M
Linoleic	0·40	$6·6 \times 10^{-7}$ M
Linolelaidic	0·25	$6·2 \times 10^{-7}$ M

*Rate of transport measured with LT2 cells grown in the absence of any exogenous fatty acid.

The experimental conditions (measurement of transport) were the same as described under 'materials and methods' with the modification that different concentrations of L-phenylalanine were used for different incubations. Incubation was carried out for 15 s at 25°C. The rate of transport is linear for this period. K_m for each set of cells was calculated from Lineweaver Bark plot.

Table 4. Lipid-protein ratio of membrane vesicles prepared from cells grown under different conditions.

Cell	Fatty acid supplementation	Growth conditions/ temperature	Protein mg/ml	Phospho-lipid mg/ml	Protein: Phospho-lipid
LT2	—	37°C	3·20	0·88	3·63 : 1
fabB2	—	30°C	3·8	1·26	3·01 : 1
fabB2	—	grown at 30°C then shifted to 37°C			
		after 1 h of shift	3·6	2·20	1·64 : 1
		after 2 h of shift	1·4	0·95	1·64 : 1
fabB2	Oleic acid	37°C	3·0	1·01	1·47 : 1
fabB2	Elaidic acid	37°C	4·0	1·94	2·06 : 1
fabB2	Palmitoleic acid	37°C	5·1	0·70	7·28 : 1
fabB2	Palmitelaidic acid	37°C	5·2	1·56	3·33 : 1
fabB2	Linoleic acid	37°C	9·0	2·63	3·42 : 1
fabB2	Linolelaidic acid	37°C	6·5	2·54	2·55 : 1

Membrane vesicles were prepared from cells grown under different supplementation or after temperature shift. Proteins as well as phospholipids were estimated as described under 'materials and methods'.
Values given are averages of 4 to 5 preparation.
Proteins and phospholipids were estimated from same samples.

linolelaidic acid the affinity of the L-phenylalanine transport system for its substrate is equal to that of corresponding *cis*-fatty acid (linoleic acid) grown cells, but the values for V_{max} differ. V_{max} obtained with linolelaidic acid grown cells is 60% of the value obtained with linoleic acid grown cells.

Effect of fatty acid supplementation on the amount of protein present in cell membrane perse

The results presented in table 3 sugest that the membrane vesicles prepared from cells grown in media supplemented with elaidic acid demonstrate very low V_{max} for phenylalanine transport but not much reduced K_m value as compared with that of the oleic acid supplemented cells. This suggested that cell membrane rich in elaidic acid contains less protein *perse.* Hence lipid-protein ratio of the cell membrane grown under our experimental condition was measured. Results presented in table 4 clearly indicate that the protein: lipid ratio is lower in case of *trans*-unsaturated fatty acid grown cells. This is specially remarkable in the case of elaidic acid supplemented cell. Protein: lipid ratio of the membrane vesicles grown in the presence of elaidic acid resemble very much that of the cells which have been transferred to nonpermissive temperature (37°C) from permissive temperature (30°C). It is remarkable that the two conditions as described above *i. e.* growth in the presence of elaidic acid and temperature shift are physiologically and biochemically very similar and support our observation reported earlier (Deb *et al.,* 1986). It may be discussed here that the activity of D-lactate dehydrogenase, a membrane bound enzyme also varies with variation in fatty acid supplementation and corraborates our finding on the gross lipid protein composition of the membrane. Cells grown in the presence of elaidic acid as well as cells shifted to 37°C exhibited very low D-lactate dehydrogenase activity about l/4th to 1/10th of the activity present in LT2 strain grown at 37°C (data not given; will be published later in detail). The protein synthesis in general is not much affected in elaidic acid supplemented cells. The generation time of fab B2 in elaidic acid supplemented medium is longest. The value increases from 75 min (under palmitoleic acid supplementation, the shortest generation time) to 96 min in the presence of elaidic acid (Deb *et al.,* 1986). Such a change in the generation time cannot account for the extent of inhibition in protein synthesis in general resulting 30 fold differences in $V_{max.}$ The results documented above suggest that membrane lipids influence the membrane function in various ways, either by changing the affinity of membrane protein or by affecting the assembly of the proteins in the lipid matrix.

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References

Bligh, E. G. and Dyer, W. J. (1959) *Can. J. Biochem. Physiol.,* **37,** 911.
Braford, M. M. (1976) *Anal. Biochem.,* **72,** 248.
Chakravorty, M. (1970) *J. Virol.,* **5,** 541.
Chen, P. S., Toribara, T. Y. and Warner, H. (1956) *Anal. Biochem.,* **28,** 1756.
Cronan, J. E. Jr. (1978) *Annu. Rev. Biochem.,* **47,** 163.

Deb, J. K., Biswas, S. K., Chakrabarti, P. and Chakravorty, M. (1986) *J. Biosci.,* **10,** 335.
Endo, A. and Rothfield, L. I. (1969) *Biochemistry,* **8,** 3508.
Esfahani, M., Rudkin, B. B., Cutler, C. J. and Waldron, P. E. (1977) *J. Biol. Chem.,* **252,** 3194.
Higashi, Y. and Strominger, J. L. (1970) *J. Biol. Chem.,* **245,** 3691.
Hofee, P., Engelsberg, E. and Lamy, F. (1964) *Biochim. Biophys. Acta,* **79,** 337.
Hong, J. S. and Ames, B. N. (1971) *Proc. Natl. Acad. Sci. USA,* **68,** 3158.
Jakovcic, S., Getz, G. S., Rabinowitz, M., Jakob, H. and Swift, S. (1971) *J. Cell. Biol.,* **48,** 490.
Kundig, W. and Roseman, S. (1975) *J. Biol. Chem.,* **246,** 1407.
Khandekar, P. S., Burma, D. P., Taneja, S. and Chakravorty, M. (1975) *J. Virol.,* **16,** 101.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.,* **193,** 265.
Mandal, S. B., Sen, P. C, Chakrabarti, P. and Sen, K. (1978) *Can. J. Microbiol.,* **24,** 586.
Milner, L. S. and Kaback, H. R. (1970) *Proc. Natl. Acad. Sci. USA,* **66,** 1008.
Ovarth, P., Schairer, H. M. and Stoffel, W. (1970) *Proc. Natl. Acad. Sci. USA,* **67,** 606.
Schneider, E. G. and Kennedy, E. P. (1973) *J. Biol. Chem.,* **248,** 3739.
Silbert, D. F. (1975) *Annu. Rev. Biochem.,* **44,** 315.